GYN Cytology

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Cervical Cytology Preparations

Conventional  ThinPrep, 20mm  SurePath, 13mm

Liquid Based Preparations

<table>
<thead>
<tr>
<th>ThinPrep</th>
<th>SurePath</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flatter prep/less dense cellularity</td>
<td>3 dimensionality/dense cellularity</td>
</tr>
<tr>
<td>Bloody specimens</td>
<td>Reduction of blood and inflammation</td>
</tr>
<tr>
<td>Lubricant issues</td>
<td>Ethanol fixative</td>
</tr>
<tr>
<td>Methanol fixative</td>
<td>HC2 - not FDA approved</td>
</tr>
<tr>
<td>HC2 - FDA approved</td>
<td>HC2 - FDA approved but validated</td>
</tr>
</tbody>
</table>

The 2001 Bethesda System

- Specimen Type
- **Specimen Adequacy**
  - General Categorization (optional)
  - Interpretation/Result
    - Negative for Intraepithelial Lesion or Malignancy (NILM)
    - Other
    - Epithelial Cell Abnormality
    - Other Malignant Neoplasms
  - Ancillary Testing
  - Automated Review
  - Educational Notes and Suggestions (optional)

Specimen Adequacy Categories

- **Satisfactory for evaluation:**
  - Describe presence of TZ component and any other quality indicators (QIs) e.g. blood, inflammation

- **Unsatisfactory for evaluation (specify reason...)**
  - Specimen processed and examined, but unsat for evaluation of epithelial abnormality. CAP 50th % 1% CP/TP and 0.3% SP
  - Specimen rejected / not processed because of ...

Definition of Cellularity

- **Conventional Pap**
  - 8-12,000 well-preserved & visualized squamous cells and squamous metaplastic cells
  - Range should be estimated
  - Use visual reference images (4x)

- **Liquid Based Prep:**
  - Minimum 5,000 squamous cells
  - Count 10 fields at 40X along center diameter
  - Imager/FOVs
  - Consider “holes” in overall cell count
  - Determine if limitation is technical in nature and reprocess if warranted

TBS 2001
<table>
<thead>
<tr>
<th></th>
<th>BD SurePath</th>
<th>ThinPrep</th>
</tr>
</thead>
<tbody>
<tr>
<td>cells for 5K FN 0 eyepiece 10X</td>
<td>118.3</td>
<td>50</td>
</tr>
<tr>
<td>cells for 5K FN 0 eyepiece 40X</td>
<td>7.4</td>
<td>3.1</td>
</tr>
<tr>
<td>cells for 5K FN 2 eyepiece 10X</td>
<td>143.2</td>
<td>60.5</td>
</tr>
<tr>
<td>cells for 5K FN 2 eyepiece 40X</td>
<td>9.0</td>
<td>3.8</td>
</tr>
</tbody>
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Endocervical Component: Management

• NILM, No ECC = Satisfactory Pap
  – 12 month follow-up

• History abnormal pap, incomplete visualization of cervix, immunocompromised status, or poor screening history, repeat Pap in 6 months


Endocervical Component: Atrophy

• Parabasal type cells may mimic squamous metaplasia and small columnar cells
• Post partum and progesterone may produce similar patterns as atrophy

  – Only use squamous metaplasia as an ECC indicator if they can be definitively identified
  – Comment on hormonal pattern inhibiting the ability to determine the presence of an ECC

Obscuring Factors: Quality Indicators

Satisfactory with QI 50% to 75%   Unsatisfactory >75%

Reprocessing unsatisfactory TP specimens with GAA can substantially reduce the overall unsatisfactory rate and result in detection of significant lesion
TP Lubricant Use

lubricants that do not contain interfering substances:
• KY Jelly
• Surgilube
• Astroglide
• Crystelle

“Any specimen with abnormal cells is by definition satisfactory for evaluation.”

• If unsatisfactory, still:
  – Indicate organism, endometrials over 40 etc
  – May contain a higher rate of cancers or SIL on follow up specimens because of obscuring of a lesion by blood or inflammation.

The 2001 Bethesda System

• Specimen Type
• Specimen Adequacy
• General Categorization (optional)
• Interpretation/Result
  – NILM: Organisms/ Other Nonneoplastic Findings
  – Epithelial Cell Abnormality
  – Other Malignant Neoplasms
• Ancillary Testing
• Automated Review
• Educational Notes and Suggestions (optional)

Reactive cellular changes associated with inflammation (includes typical repair)

• Bland nuclei > 1.5 to 2 X area of intermediate cell nucleus
• Bi and multi-nucleation.
• Round and uniform nuclei, smooth nuclear outlines.
• Mild hyperchromasia, even distribution.
• Nucleoli may be present
• Cytoplasmic polychromasia, vacuolization, halos

Reactive cellular changes associated with radiation/chemotherapy and FA deficiency

• Cytomegaly without an increase in N:C ratio
• Cytoplasmic vacuolization/polychromasia
• Coexisting changes of repair
Reactive cellular changes associated with IUD

- Few glandular cells with large cytoplasmic vacuoles
- May have high N:C ratios and/or nucleoli
- Calcifications/Actin

Atrophy

- Flat sheets of parabasal cells with preserved nuclear polarity
- Nuclei may be elongated with uniform chromatin distribution
- Giant cells
- Dirty background
- Transitional metaplasia

Hyperkeratosis (HK) and Parakeratosis (PK)

- Prolapse, inflammation, pessary, XRT
- Little clinical significance when limited
- May be seen overlying SIL
- Abundant clusters may be significant
- Follow closely

Follicular/Lymphocytic Cervicitis

- Polymorphous population of lymphocytes with or without tingible body macrophages
- Some are related to Chlamydia infection

TBS: Epithelial Cell Abnormalities: Squamous cell

- Atypical squamous cells (ASC)
  - of undetermined significance (ASC-US)
  - cannot exclude HSIL (ASC-H)
- Low-grade squamous intraepithelial lesion (LSIL) encompassing human papillomavirus/mild dysplasia/cervical intraepithelial neoplasia (CIN) 1
- High-grade squamous intraepithelial lesion (HSIL) encompassing: moderate and severe dysplasia, carcinoma in situ, CIN 2 and CIN 3
- Squamous cell carcinoma

Size relationships (Benchmarks)

- Cell size and shape
- Position of nucleus
- N:C ratio
- Chromatin Pattern
- Presence of nucleoli
- Cytoplasm
- Cell arrangements
- Background information
- Intermediate cell
- Squamous metaplasia
- Reactive/ASC
- ASC-US
- SIL
Atypical Squamous Cells (ASC)

ASC-US: An ill-defined category created to address the inherent limitations of cytologic examination in the distinction of subtle cytologic changes.

Atypical Squamous Cells (ASC): Cytologic changes suggestive of a squamous intraepithelial lesion that are quantitatively or qualitatively insufficient for a definitive interpretation.

ASC-US: Cytologic Features

GENERAL:
• Cells are usually isolated
• Number of affected cells low (3-5)
• Cytoplasmic area approximate superficial or intermediate squamous cells in most instances and less often squamous metaplastic cells
• Absence of Human Papillomavirus (HPV) cytopathic effect

NUCLEAR:
• Area 2-3x size (2.5-3.5x) of intermediate cell nucleus or 2x nucleus of immature squamous metaplastic cell
• Limited variation in nuclear size & shape
• Nuclei are round and usually have smooth nuclear envelopes. Very limited nuclear membrane irregularity.
• Minimal nuclear hyperchromasia

ASC-US: Not a Single Entity

• Atypical cells with “mature” intermediate type cytoplasm (NOS)
• Atypical parakeratotic squamous cells (AP)
• Atypical repair
• Atypical cells in the setting of atrophy
• Atypical cells due to compromised specimen

ASC-US: Not a Single Entity

• The ASC category was developed to designate equivocal specimens not individual cells
• Does not represent a single biologic entity
• Subsumes changes unrelated to oncogenic HPV infection and neoplasia as well as those of SIL, and rarely carcinoma
• ASC may assume infinite appearances
ASC-US: Not a Single Entity

- Atypical cells with equivocal changes of HPV
- Atypical cells due to compromised specimen
- Atypical cells in setting of inflammation

ASC-H: Changes that are suggestive of HSIL but lack criteria required for a definitive interpretation (5-10% of ASC)

- Sparse “atypical immature metaplastic cells”
- Singly or in tight small clusters (<10 cells)
- Slight increase in N/C ratio (usually ≥50%)
- Nuclear abnormalities
  – Mild irregular nuclear contours, mild hyperchromasia
  – Can be subtle

ASC-H: Not Synonymous with HSIL

- Not a “biologic” or “morphologic” entity.
- PPV for high grade lesion (≥ CIN 2) is greater than ASC-US, but less than HSIL
- Should be investigated like HSIL, but if CIN2/3 is not confirmed by histology, may be spared aggressive treatment.
- Encourage pathologists to correlate cytology and histology if ≥CIN2 is not confirmed.

ASC-H HSIL

- Increased N/C ratio (>75%). Irregular nuclear contours. Coarse chromatin
  - Numerous small atypical cells and clusters

Sources of Histologic CIN 2+

- ASC-US: 4-5% of all cytologic interpretations and most common diagnosed cervical abnormality.
- Most common Pap interpretation that preceded a biopsy on CIN2+
**ASC-US**

- Few cells
- Slight nuclear enlargement (x2-3 intermediate cell nucleus)
- Stippled chromatin
- Regular nuclear membranes

ASC-US < 5% of Paps
ASC/SIL ratio < 3.1 (1.4-2)

**LSIL**

- Multiple cells
- Enlarged nucleus (>x3 intermediate cell nucleus)
- Smudgy or hyperchromatic nuclei
- Nuclear membrane irregularities
- Koilocytes

**LSIL**

**GENERAL:**

- Cells occur singly or in sheets
- Cytoplasmic maturation with distinct borders
- Koilocytes:
  - Perinuclear cavity: Sharply cut borders,
  - Various degrees of nuclear atypia
  /degeneration

**LSIL**

**NUCLEUS:**

- 3-6x size of ICN or 2-4x size of MCN
- Moderate variation in nuclear size
- Mild nuclear hyperchromasia
- Mild nuclear irregularities
- Chromatin finely granular and uniformly distributed
- Nucleoli are absent
- Binucleation/multinucleation.

**LSIL Cannot Rule Out HSIL (LSIL-H)**

- Majority of cells are LSIL, few are ASC-H
- LSIL-H has slightly greater likelihood of underlying CIN-2+ (2 years) than LSIL (10.8%) and similar likelihood of CIN2/3+ as ASC- H (27%).
- Controversial as LSIL has built in a % of CIN2+ but
  - more sampling at colposcopy
  - deeper levels obtained by pathologist

**HSIL**

**GENERAL:**

- Isolated cells and cell aggregates (syncytial-like arrangements)
- Immature cytoplasmic characteristics or pleomorphic keratinized configurations

**HSIL**

**NUCLEUS:**

- Decreased nuclear size and marked increase in N/C ratio
- Nuclear hyperchromasia
- Chromatin clumping/oarsening of chromatin
- Irregularities of nuclear outlines
**HSIL: Differential**

- ASC-H (SP)
- Endometrial stromal cells

**HPV Prevalence**

1. HR-HPV positive in:
   - 95% HSIL/Carcinoma
   - 75-85% LSIL
   - 70-85% ASC-H
   - 50% ASCUS
   - Variable % normal Paps <10% (10-20%)

HPV-16 and HPV-18 are associated with 70% of all invasive carcinomas.

**HPV testing provides effective triage for ASC-US.**

2. HR-HPV test captures virtually all CIN 2+:
   - Cumulative probability of HSIL/CIN 2+ in 2 years ASC-US HPV positive is 25-30%
     (12-17% at incident colposcopy)
   - The negative predictive value for the HPV test is 99% (98.9-99.3%).

**HPV Testing and Management Guidelines**

- HPV “Reflex” testing for equivocal ASC-US Cytology:
  - LSIL is associated with HR-HPV and is not amenable to triage:
  - Post colposcopy follow up of woman with abnormal cytology found to have less than a high grade lesion.


- Triage or follow up with HPV DNA testing is not recommended for ASC-US in adolescents.
- LSIL and ASC-US/HPV positive are managed similarly.
- ASC-H necessitates colposcopy/biopsy.
- For pregnant women the only indication for therapy is invasive cancer.
- HSIL necessitates colposcopy/biopsy.
**Squamous Cell Carcinoma**

- Greater depth-to-focus of cell groups
- Nuclear characteristics of malignancy
- Course irregular chromatin
- Nucleoli
- Irregular cytoplasmic shapes and conformations

**Small Cell Carcinoma:**

- Single cells and syncytial groups of small, cuboidal or round cells
- High N/C ratio with scant cyanophilic cytoplasm
- Large, round to oval nuclei with hyperchromatic, coarsely granular chromatin
- Nuclear molding and small nucleoli

**Squamous cell carcinoma: Tumor diathesis**

- Greater depth-to-focus of cell groups
- Nuclear characteristics of malignancy
- Course irregular chromatin
- Nucleoli
- Irregular cytoplasmic shapes and conformations

**TBS: Epithelial Cell Abnormalities: Glandular cell**

- Atypical
  - Endocervical cells (NOS or specify in comments)
  - Endocervical cells, favor neoplastic
- Endometrial cells (NOS or specify in comments)
- Glandular cells (NOS or specify in comments)
- Glandular cells, favor neoplastic
- Endocervical adenocarcinoma in situ
- Adenocarcinoma
  - Endocervical
  - Endometrial
  - Extrauterine
  - Not otherwise specified (NOS)

Note: CIN is the most common significant finding identified in women with AGC. CIN lesions have been found in 8% to 83% of women with AGC, of which 40% to 68% are CIN II.

**Hyperchromatic crowded group:** Grouping with features that impede the ability of the cytologist to see the individual cells in the middle

- Benign
  - Endocervical cells
  - Endometrial cells
  - LUS
  - Atrophy
  - Tubal metaplasia
  - Microglandular hyperplasia
  - Clusters of inflammatory cells
- Neoplastic/Preneoplastic
  - HSIL
  - AIS
  - Squamous cell carcinoma
- Adenocarcinomas

**EC Adenocarcinoma in Situ (AIS)**

1. Hyperchromatic crowded groups
2. Increased N/C ratio
3. Nuclei large (75 um2)
4. Even chromatin coarse granularity
5. Micronucleoli
6. Rosettes
7. Feathering
8. Strips with pseudostratification
9. Mitoses, apoptotic bodies
10. Amphophilic granular cytoplasm
11. Clean/inflammatory background
**EC Adenocarcinoma in Situ**

1. Abundant Cellularity
2. Flat or three D Sheets
3. Features of AIS
4. Single cells
5. Macro nucleoli
6. Decreased, finely vacuolated cytoplasm

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**Endocervical Adenocarcinoma**

1. Increased N/C ratio
2. Pleomorphic nuclei, nuclear irregularity, unevenly distributed chromatin/chromatin clearing.
3. Necrotic tumor diathesis

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**Endocervical Adenocarcinoma**

• Pseudostratified, crowded groups of endocervical cells
• No nucleoli
• Cilia/terminal bars

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**Tubal Metaplasia**

- Report in all women 40 or older (0.5% to 1.8% of Paps).
- Tight 3-D clusters, loose clusters, or single cells.
- Bean-shaped nuclei. Nucleoli and chromatin pattern more apparent, cytoplasmic vacuoles.

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**Endometrial Adenocarcinoma**

- Single or small loose or tight clusters.
- Nuclear size increase with grade of the tumor.
- Variation in size and shape, unevenly distributed chromation.
- Nucleoli
- Small amount of vacuolated cytoplasm/intracytoplasmic neutrophils

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**Endometrial Cells**

- Increased N/C ratio
- Pleomorphic nuclei, nuclear irregularity, unevenly distributed chromatin/chromatin clearing.
- Necrotic tumor diathesis
Endometrial Adenocarcinoma and Metastatic Disease

ASCCP: 2006 Guidelines (www.asccp.org)

- Colposcopy with endocervical sampling is recommended for all subcategories of AGC. Endometrial sampling is added for AGC >35 years.
- Cotesting for Paps > 30 years, guidelines for women (30 y and older) who test negative for HR-HPV and the role HPV-16 and 18 genotyping.

Trichomonas Vaginalis

- Pear-shaped (15-30 µm)
- Vesicular eosinophilic eccentric nucleus
- Cytoplasmic granules
- Dirty background.
- Flagella may be seen
- Cannonballs of inflammatory cells
- Pseudoeosinophilia, perinuclear halos and mildly enlarged nuclei.

Fungal organisms morphologically consistent with Candida spp.

- Budding yeast
- 3-7 microns
- Pseudohyphae
- Fragmented polys and rouleau formation
- Spearing prominent pattern

- Mild nuclear enlargement and hyperchromasia
- Mimics ASCUS

Shift in flora suggestive of bacterial vaginosis (BV)

- Absence of lactobacilli
- Polymicrobial, as a synergistic infection with Gardnerella vaginalis and one or more anaerobic bacteria: Mobiluncus, Peptococcus or Bacteroides.
- Clue cells are fairly predictive of vaginosis

Clinical correlation (Vag pH, wet prep, "whiff" test on KOH preparation, cultures)

Bacteria morphologically consistent with Actinomyces spp.

- Tangled clumps of filamentous organisms
- Acute angled branching
- Cotton-ball cluster
- Radial distribution of filaments.
- Acute inflammation.

- Associated with IUD use
- May alert clinician to evidence of pelvic infection
Cellular changes consistent with herpes simplex virus

- “Ground-Glass” nuclei
- Dense eosinophilic intranuclear inclusion surrounded by a halo may be present
- Multinucleated epithelial cells with molded nuclei may be present.
- Single cells of herpes may mimic dysplasia

Artifacts
- Colonic mucosa
- Pollen
- Corn flakes
- Plant
- Sperms

Additional Artifacts
- Alternaria
- Decidual cells
- Trophoblasts

Thank You